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(54) Title: ANIMAL AND CELL MODELS FOR TYPE II DIABETES AND THEIR USE

(57) Abstract: A transgenic diabetes type II model laboratory animal is disclosed which comprises β-cells expressing a dominant negative form (dnFGFR1c) of FGFR1c. Also disclosed is the use of the *Ipf1/Pdx1* promoter for controlling expression of FGFR1c; β-cells in wich the expression of PC1/3 is down-regulated or absent or which are competent to express a dominant negative form (dnFGFR1c) of FGFR1c; mature β-cells incompetent to express Glut2 or in which the processing of proinsulin is substantially impaired; a method of preventing or treating type II diabetes.

ANIMAL AND CELL MODELS FOR TYPE II DIABETES AND THEIR USE

FIELD OF THE INVENTION

The present invention relates to an animal model for type II diabetes, in particular a transgenic mouse, in which the expression of fibroblast growth factor receptors essential for ensuring a functional ß-cell identity is perturbed. The invention also relates to the use of this model for studying type II diabetes, in particular with the aim of developing therapies therefore. The invention also relates to a corresponding cells and their components useful as in-vitro or in-vivo models. Furthermore the invention relates to a method of preventing or treating diabetes type-II.

BACKGROUND OF THE INVENTION

The Fibroblast Growth Factor (FGF) gene superfamily is a family of conserved, secreted proteins that have been shown to play a critical role in many biological processes (Kato and Sekine, 1999; Szebenyi and Fallon, 1999). FGF-signalling is achieved by binding of the ligand, FGF, to the extra-cellular domain of high affinity membrane bound FGFR, which belongs to the tyrosine kinase family of receptors (Kato and Sekine, 1999; Szebenyi and Fallon, 1999). Today around 20 different FGF genes and 4 different FGFR genes have been identified, and multiple ligands can interact with one and the same receptor (Kato and Sekine, 1999; Szebenyi and Fallon, 1999). The level of complexity of signalling via these receptors is further compounded by the fact that alternative splice variants exist for these receptors. Loop three of the extracellular domain (=ligand binding domain) can splice to give rise to b, or c, isoform. This isoform variation ultimately determines ligand specificity and proper

ligand-receptor interaction ultimately leads to activation of the intracellular tyrosine kinase domain (Kato and Sekine, 1999; Szebenyi and Fallon, 1999).

FGF-signalling has been implicated in a variety of distinct biological processes including patterning, differentiation, morphogenesis, proliferation, survival, angiogenesis, tumorogenesis, etc. (Kato and Sekine, 1999; Szebenyi and Fallon, 1999). In mouse, an early embryonic lethality or functional redundancy have, however, largely hampered direct genetic approaches aiming at elucidating the role of FGF-signalling during development and in the adult. Thus these approaches has for the most part failed to provide critical information regarding the role of FGF-signalling during later stages of vertebrate organogenesis, including the pancreas. An alternative approach have been to impair FGF-signalling via organ specific expression of dominant negative forms of FGFR that will competitively block FGF signalling via the endogenous, corresponding FGFR variant. This approach has been successfully used to antagonise FGF-signalling in a number of different systems.

Viral infection of a dominant negative FGFR1 construct in chick limb muscle mass blocked the differentiation of myoblast to myotubes providing evidence that this process depends on FGF-signalling (Itoh et al., 1996). Studies focused on maintenance of cell types within the retina revealed that expression of dnFGFR2 under the control of the bovine rhodopsin promoter increased photoreceptor degeneration (Campochiaro et al. 1996). The specificity of dominant negative constructs with respect to ligand binding was demonstrated in analyses where dnFGFR1c and dnFGFR2b constructs where expressed in transgenic mice using the mammary tumour virus promoter (Jackson et al.,1997).

Expression of dnFGFR1c under these conditions did not result in any discernible phenotype whereas an impairment of lobuloalveolar development in the mammary gland was observed when using the dnFGFR2b variant (Jackson et al.,1997). Moreover, FGF8 mediated induction of dopaminergic (DA) neurons was successfully inhibited when growing six somite rat ventral mid/hindbrain explants in presence of soluble dnFGFR3c, i.e. the high-affinity blocking receptor for FGF8 (Ye et al., 1998). In contrast, when the same experiment was performed using soluble, dnGFGR1c, a low-affinity nonblocking receptor for FGF8, DA neurons readily appeared (Ye et al., 1998). Together these analyses demonstrate the effectiveness by which FGF signalling, in an apparent ligand-specific manner, can be perturbed using a dominant-negative FGFR approach.

Three different FGF-signalling mutant mice, involving transgenic approaches to over-express either a ligand or a dn form of a receptor, resulting in a pancreatic phenotypes have been reported. Transgenic over-expression of *FGF7/KGF* in the mouse liver induced pancreatic ductual hyperplasia (Nguyen et al. 1996) and similarly, transgenic mice with forced expression of FGF-7/KGF in pancreatic β-cells under the control of the insulin promoter show enlarged islets containing proliferating duct cells (Krakowski et al., 1999). General transgenic over-expression of *dnFGFR2b* under the control of the metallothionein promoter resulted in pancreatic hypoplasia (Celli et al. 1998). Together these studies indicate that signalling through FGFR2b may operate during pancreatic development. *In vitro* experiments involving culturing of pancreatic rudiments support such a scenario and suggest that FGFs positively stimulate pancreatic epithelial cell proliferation and exocrine cell differentiation (Le Bras et al. 1998, Miralles et al. 1999).

Selective inactivation of the IIIb form of FGFR2 leads to developmental abnormalities in limbs, lung, anterior pituitary, salivary glands, inner ear, teeth and skin but apparently not in the pancreas (De Moerlooze et al., 2000). Thus, the roles of FGFR2b during pancreas development remain to be determined.

Failure of the β-cell to compensate for an increased demand for insulin is a key feature in the manifestation of type 2 diabetes. Type 2 diabetes is the most common form of diabetes, affecting 2-3% of the world-wide population, and is the combined result of resistance to insulin action coupled with a defect in β-cell compensation (Kahn, 1998; Kahn and Rossetti, 1998; Taylor, 1999). The molecular defects underlying the development of the disease are not fully understood and there are also uncertainties as to what is the primary defect initiating the disease; the insulin resistance or the β-cell failure. A typical trait associated with the disease is the increased proinsulin to insulin (P/I) ratio observed in many type 2 diabetic patients (Porte and Kahn, 1989). The relationship between the increased P/I ratio and the etiology of the disease has however remained diffuse; i.e. is it a consequence rather than a directly contributing factor to the disease? Several independent studies points towards an increased P/I ratio being an early sign of primary β-cell dysfunction, independent of insulin resistance, which is directly associated with the conversion from a prediabetic to an overt diabetic state over a short time period (Mykkänen et al., 1995; Kahn et al., 1995, Nijpels et al., 1996; Rachman et al., 1997; Mykkänen et al., 1997; Haffner et al 1997; Larsson and Ahrén, 1999). Moreover, it has been suggested that normal β-cells respond to an increased insulin resistance by enhanced processing of insulin and that the increased P/I ratio in individuals with an impaired glucose

tolerance, and/or type 2 diabetes, is the consequence of defects in proinsulin processing (Mykkänen et al., 1997, Larsson and Ahrén, 1999).

Processing of proinsulin to insulin in β-cells is catalysed by the sequential actions of prohormone convertases PC1/3 and PC2, which both act in concert with carboxpeptidase E (CPE) (Figure 7). Analyses of PC2 null mutant mice demonstrated a crucial role for PC2 in the processing of proglucagon and prosomatostatin in α- and δ- cells, respectively (Furuta et al., 1998). Proinsulin processing in β-cells was less affected in the PC2 null mutant mice providing evidence that PC3 is quantitatively more important than PC2 with respect to processing of proinsulin to active insulin (Furuta et al., 1998). At present there is a lack of genetically defined animal models that mimic these aspects of human type 2 diabetes. The importance of this disease in terms of human suffering and health care costs makes the provision of such a model an important goal.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide an animal model which mimics human type II diabetes and which can be used to develop a therapy.

It is another object of the invention to provide a method of preventing or treating type II diabetes.

Further objects of the invention will become evident from the study of the following short description of the invention and preferred embodiments thereof, the figures illustrating the invention, and the appended claims.

SUMMARY OF THE INVENTION

The invention is based on the insight that, in addition to the previously reported expression of FGFR2b during pancreas development, FGFR2 and FGFR1 are both expressed in the adult ß-cell. In regard of FGFR1, a functional role was demonstrated by impairing signalling through FGFR1c via the expression of a dominant negative form of this receptor, dnFGFR1c (Ye et al., 1998), under control of the *Ipf1/Pdx1* promoter (Apelqvist et al., 1997).

Mice expressing the dnFGFR1c exhibit a grossly normally developed pancreas with no apparent abnormalities but develop diabetes with age. The expression of dnFGFR1c in ß-cells results in disorganised islets with reduced numbers of ß-cells displaying an apparent immature molecular identity. First, the ß-cells do not express detectable of levels Glut2 which is one of the key components of the glucose sensing machinery. Secondly, the expression of one of the proinsulin processing enzymes, PC1/3, is impaired. Third, although insulin is synthesised by the ß-cells it fails to be fully processed and remains largely in the form of pro-insulin and/or partially processed.

According to the present invention, evidence is provided that the expression of FGFR1 is dependent on *Ipf1/Pdx1* expression. *Rip1/Ipf1*$^{\wedge}$ mice in which *Ipf1/Pdx1* has been inactivated selectively in ß-cells display disorganised islets and develop diabetes due to decreased insulin expression combined with a loss of Glut2 expression (Ahlgren et al., 1998). The expression of both FGFR 1 is down-regulated in the *Rip1/Ipf1*$^{\wedge}$ mice suggesting that *Ipf/Pdx1* is required for expression of *FGFR1* and *FGFR2* in ß-cells. Moreover, in the *Rip1/Ipf1*$^{\wedge}$ mice, alike in the *Ipf1/dnFGFR1c* mice, PC1/3 expression is

impaired. These results suggest that signalling via FGFR1c is required for ensuring a correct number of ß-cells and their proper function. Moreover, the results provide evidence that *Ipf1/Pdx1* controls many aspects of the ß-cell glucose homeostasis machinery, in part, by being required for the expression of *FGFR1* in the ß-cell.

In humans, heterozygosity for a nonsense mutation in the Ipf1 gene, which results in a dominant negative frameshift, has been linked to Maturity-Onset Diabetes of the Young (MODY) 4 [Stoffers et al., 1997], a monogenetic form of diabetes that results from β-cell dysfunction rather than insulin resistance. Moreover, missense mutations in the human Ipf1 gene are implicated in predisposing an individual to type 2 diabetes [Macfarlane et al., 1999; Hani et al., 1999]. Previous work has shown that Ipf1/Pdx1 is required for ensuring normal levels of insulin and Glut2 expression [Ohlsson et al., 1993; Ahlgren et al., 1998]. According to the present invention, as explained above, there is now genetic evidence suggesting that Ipf1/Pdx1 acts upstream of FGF-signalling in the β-cell, since genetic inactivation of Ipf1/Pdx1 in β-cells, as in the RIP1/Ipf1$^{\Delta}$ mice [Ahlgren et al., 1998], leads to reduced expression levels of FGFR1, and the ligands FGF1, FGF2, FGF4 and FGF5. Consequently, the RIP1/Ipf1$^{\Delta}$ mice also display reduced expression of PC1/3 paralleled by an increase in proinsulin in the β-cells of these mice.

The phenotypes observed in the FRID1 mice, i) reduced β-cell number, ii) loss of Glut2 expression leading to impaired glucose sensing and, iii) perturbed proinsulin processing due to the down regulation of prohormone convertase 1/3 and 2 expression [6], are reflective of the β-cell dysfunction

associated with type 2 diabetic patients [Porte et al., 1989; Hales, 1994; Mykkanen et al., 1995; Mykkanen et al., 1999; Kahn et al., 1995; Kahn et al., 1995 bis; Larsson et al., 1999; Nijpels et al., 1996; Rachman et al., 1997; Haffner et al., 1997]. These findings suggest that signalling via FGFR1c may represent one factor required for β-cell expansion both during early life and in response to hyperglycaemia. Morover these data provide evidence that FGFR1c-signalling in the β-cell is required to ensure normal expression of key components in glucose sensing (Glut2) and insulin processing machinery (PC1/3 and PC2) and thus to maintain normoglycaemia. Last the analyses of the RIP1/Ipf1$^{\Delta}$ mice provide genetic evidence that that the IPF1/PDX1 transcription factor acts upstream of FGFR1-signalling in controlling key aspects of β-cell identity. The apparent conservation of Ipf1/Pdx1 gene function from mice to humans suggest that also the downstreams effects controlled by Ipf1/Pdx1 gene activity may be conserved [Stoffers et al., 1997; Macfarlane et al, 1999; Hani et al., 1999; Ohlsson et al., 1993; Ahlgren et al., 1998]. This strongly suggests that that FGF-signalling is important for β-cell function also in humans and that pertubation of this signalling pathway in adult human β-cells is linked to type II diabetes.

According to the present invention thus is disclosed a transgenic diabetes type II model laboratory animal comprising ß-cells expressing a dominant negative form (dnFGFR1c) of FGFR1c. In particular the transgenic animal is a mouse.

According to the present invention is also disclosed the use of the *Ipf1/Pdx1* promoter for controlling the expression of FGFR1c.

According to a first preferred aspect of the invention are disclosed ß-cells in which the expression of PC1/3 is down-regulated or absent. Preferably the ß-cells are comprised by an adult pancreas.

According to a second preferred aspect of the invention are disclosed ß-Cells competent to express a dominant negative form (dnFGFR1c) of FGFR1c. Preferably the ß-cells are comprised by an adult pancreas.

According to a third preferred aspect of the invention are disclosed mature ß-cells incompetent to express Glut2. Preferably the ß-cells are comprised by an adult pancreas.

According to a fourth preferred aspect of the invention are disclosed mature ß-cells in which the processing of proinsulin to insulin is substantially impaired. In these cells levels of proinsulin convertase 1/3 are substantially reduced in comparison with the levels in non-transgenic mice. Preferably these ß-cells are comprised by an adult pancreas.

According to a fifth preferred aspect of the invention are disclosed a reduced number of ß-cells and a failure of ß-cells to respond to hyperglycemia by replication.

In the following the invention will be by described in more detail by reference to preferred but not limiting embodiments.

SHORT DESCRIPTION OF THE FIGURES

The preferred embodiments are illustrated by a number of figures showing:

Fig. 1 expression of FGFR1 in adult β-cells;

Fig. 2 reduction in number of ins^{+}-cells in *Ipf1/dnFGFR1c* mice;

Fig. 3 defects in β-cell identity in *Ipf1/dnFGFR1c* mice;

Fig. 4 impaired pro-insulin processing in *Ipf1/dnFGFR1c* mice;

Fig. 5 control by pf1/Pdx1 of multiple aspects of β-cell identity including FGFR1 expression.

DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1. Expression of FGFR1 in the adult pancreas.

Analysis of FGFR1 expression in the adult mouse pancreas revealed that FGFR1 is predominantly expressed in the adult ß-cell, with no expression observed in the glucagon-producing α-cells (Figure 1). A lower level of FGFR1 expression was also observed in the exocrine cells of the pancreas (data not shown). FGFR2 is also selectively expressed in the adult ß-cells but in contrast to FGFR1, FGFR2 expression was not observed in the exocrine cells of the adult pancreas (data not shown). The expression of FGFR in adult ß-cells suggests a role for FGF-signalling in terminal differentiation and/or maturation of these cells.

The expression of FGFR1 in the pancreas led us to examine whether signalling via this receptor may be required for pancreas development, as has already been implied for FGFR2b. We also examined whether FGF receptor signalling was required for the specification and differentiation of adult ß-cells. To begin to address this issue we generated transgenic mice expressing dominant negative FGFR1c construct in the pancreas using the *Ipf1/Pdx1* promoter. This consisted of the three loop extracellular domain of *FGFR1c* fused in frame with the *rat IgG Fc* region. In the resulting mice Ipf1-expressing cells would secrete the hybrid protein from the cell allowing

competitive binding of FGF ligands such as FGF1, FGF2, FGF4, FGF5 and FGF6 which are known to bind to the FGFR1c variant.

Example 2. Development diabetes in *Ipf1/dnFGFR1c* mice.

Ipf1/dnFGFR1c express high levels of the transgene in ß-cells with lower levels of the transgene being expressed in the exocrine part of the pancreas (Figure 1 and data not shown). The *Ipf1/dnFGFR1c* transgenic mice are viable and fertile with a grossly well developed pancreas, providing evidence that signalling via FGFR1c is not important for pancreas growth, morphogenesis and differentiation. The mice appeared healthy until approximately 15 weeks of age when elevated non-fasting urine glucose concentrations greater than 2% were observed suggesting a diabetic phenotype. Fasting blood glucose measurements on mice were >20mM, confirming that these animals were severely diabetic.

Close monitoring of urine and blood glucose levels revealed that already at 3 weeks of age *Ipf1/dnFGFR1c* mice showed elevated glucose levels, albeit still within the normal range (Table 1). At six weeks of age their fasting blood glucose levels had increased by 1 mM and they had detectable levels of glucose in their urine when compared to wild type age matched littermates (Table 1). Weekly monitoring of the urine glucose revealed a steady increase in glucose and by the age of 9-12 weeks urine glucose levels were in excess of 2%. Non-fasting and fasting blood glucose measurements taken at this stage revealed 4-fold higher glucose levels compared with age-matched wild type littermates (Table 1). These findings demonstrate that impaired FGFR1c-signaling in adult ß-cells results in the development of diabetes and points to a crucial, hitherto unknown role for FGF-signalling in ß-cell glucose homeostasis.

Table 1. ***Ipf1/dnFGFR1c*** **transgenic mice develop diabetes**

	Blood glucose levels (mM +/- S.E.M.)	
	non-fasted	fasted
Wild type (3 weeks old)	nd	4.4+/-0.4 (n=5)
Ipf1/dnFGFR1c (3 weeks old)	nd	5.8+/-0.5 (n=7)
Wild type (6 weeks old)	nd	4.9+/-0.3 (n=5)
Ipf1/dnFGFR1c (6 weeks old)	nd	6.8+/-0.8 (n=7)
Wild type (12 weeks old)	8.7+/-0.8 (n=5)	4.0+/-0.6 (n=5)
Ipf1/dnFGFR1c (12 weeks old)	26.4+/-1.5 (n=5)	15.8+/-1.3 (n=7)

Legend to Table 1: Blood glucose levels were measured in *Ipf1/dnFGFR1c* mice and wild type littermates at the time points shown. At 3 weeks of age *Ipf1/dnFGFR1c* mice showed non-fasted blood glucose levels within the normal range. Six-week old *Ipf1/dnFGFR1c* mice had slightly elevated non-fasted blood glucose levels, still within the normal range albeit at the upper level. Overt diabetes (OD) develops in 9-12 weeks old mice in whom both non-fasted and fasted blood glucose levels were 4-fold higher than wild type, age-matched littermates.

Example 3. Demonstration of disorganized islets with reduced numbers of ß-cells in *Ipf1/dnFGFR1c* mice. The overtly normal development of the pancreas in *Ipf1/dnFGFR1c* mice homozygous mutants suggested that the growth and differentiation of the pancreas is independent of FGFR1c-signaling. To assess this further we analyzed the expression of the transcription factors Isl1, Ipf1/Pdx1, Nkx6.1 and Nkx2.2, the endocrine hormones insulin (Ins), glucagon (Glu), somatostatin (Som), and the exocrine enzymes amylase and carboxypeptidase A. Each of these markers were expressed in the pancreas of *Ipf1/dnFGFR1c* mice and the organization of endocrine cells

into islet-like clusters and of exocrine cells into acinar-like structures appeared normal (Data not shown). Nevertheless, as revealed by double immunohistochemical analysis, there was a 35% decrease in the total number of $Isl1^+$ cells paralleled by ~30% net decrease in the number of Ins^+-cells and a concomitant 20% increase in the relative number of Glu^+-cells (Figure 2). These results suggest that the genesis and/or survival of ß-cells partly depends on FGFR1c-signalling. Tunnel assays failed, however, to detect any increased ß-cell apoptosis suggesting that the decreased number of ß-cells are not caused by ß-cell death (data not shown). The 30% decrease in total number of insulin cells in the transgenic mice was reflected by a 28% decrease in total pancreatic insulin content (Figure 2). Moreover, although islets form in the *Ipf1/dnFGFR1c* mice the typical structure of maturing islets with α-cells at the periphery surrounding a core of ß-cells is perturbed; instead, Glu^+ cells are found scattered throughout the islets (Figure 3). In combination these histological analyses support the idea that the development of the pancreas to generate both exocrine and endocrine cells is unaffected despite the expression of a dn form of FGFR1c during pancreas development and in the adult ß-cell. In addition these results provide evidence of the genesis of pancreatic ß-cells appearing to be partly dependent on, and that normal organization of islet-cells requires, FGFR1c-signaling.

Example 4. Down-regulation of glucose transporter type 2 is in *Ipf1/dnFGFR1c* mice. The decrease in total insulin production by 28 % appears unlikely to be sufficient to cause the diabetes observed in the transgenic mice and suggests that additional complications underlie the development of the diabetic phenotype observed in the *Ipf1/dnFGFR1c* mice. To determine whether other key characteristics of the adult ß-

cell were affected in the transgenic mice we next monitored the expression of factors crucially required for normal glucose homeostasis. Glut2 is a key component in glucose sensing machinery within the ß-cell. Analyses revealed that the expression of Glut2 was virtually lost in overt diabetic *Ipf1/dnFGFR1c* mice (Figure 3). To exclude that the loss of Glut2 expression was a consequence of the hyperglycemic state rather than a direct effect of the *Ipf1/dnFGFR1c* transgene expression, 5-week old, prediabetic transgenic mice were analyzed. Prediabetic, 5 week old *Ipf1/dnFGFR1c* mice exhibit a clearly reduced level of Glut2 expression as compared to wild type (not shown). These results indicate that the reduction of Glut2 expression observed in *Ipf1/dnFGFR1c* mice is a direct consequence of impaired FGFR1c signalling.

Example 5. Down-regulation of PC1/3 in *Ipf1/dnFGFR1c* mice. Although the loss of Glut2 may be sufficient to cause their diabetic phenotype, the severity of the ensuing hyperglycemia in a short period of time suggested that there might be additional defects in the ß-cell of *Ipf1/dnFGFR1c* mice. Type 2 diabetes patients and animal models of the disease, often suffer from hyperproinsulinemia, reflecting an impaired processing of proinsulin to mature, active insulin which is believed to be a major contributing factor to their disease. To elucidate a potential processing defect in the Ipf1/FGFR1dn mice we performed an immunohistochemical analysis for investigating the expression of the proinsulin processing enzymes, PC1/3 and PC2. PC1/3 expression was found to be severely down-regulated so as to be virtually absent in the *Ipf1/dnFGFR1c* mice, whereas only a minor decrease was observed with respect to PC2 expression in overt diabetic mice (Figure 3 and data not shown).

Next we wanted to determine whether this aberrant processing enzymes expression could be directly involved in the development of the diabetic phenotype in the *Ipf1/dnFGFR1c* mice. To this effect pancreas from five-week old prediabetic and wild type littermates were analyzed for PC1/3 and PC2 expression. The analyses showed that already at 5-weeks of age expression levels of the prohormone convertases was reduced in the *Ipf1/dnFGFR1c* mice as compared to controls (not shown). Together, these results suggest that FGFR1c signaling is required for high level expression of PC1/3, and to a lesser extent for PC2 expression.

Example 6. Demonstration of impaired proinsulin processing in *Ipf1/dnFGFR1c* mice. To address whether the impaired expression of the processing enzymes might affect insulin processing in the *Ipf1/dnFGFR1c* mice we examined the type of insulin made and stored in the ß-cells. An antibody directed against intact human proinsulin, which do not cross-react with active insulin (Madsen et al., 1983; Madsen et al., 1984; Furuta et al., 1998), and antibodies directed against mouse C-peptide 1 and 2 (Blume et al., 1992) were used to evaluate the forms of insulin present in the β-cells of *Ipf1/dnFGFR1c* mice.

There was a marked reduction in the levels of both C-peptide 1 and C-peptide 2 in the ß-cells of the overt diabetic transgenic mice as compared to ß-cells of wild type mice (Fig 4). In contrast, high levels of proinsulin was observed in the ß-cells of the transgenic mice while no or very little proinsulin was observed in the wild type ß-cells (Figure 4). Notably increased proinsulin levels were manifest already at the prediabetic 5-week stage (data not shown). These results indicate that the down regulation of the processing enzyme, PC1/3 in the *Ipf1/dnFGFR1c* mice results in an impaired

processing of proinsulin to its active mature form. This processing defect is likely to contribute to the development of severe diabetes in the *Ipf1/dnFGFR1c* mice. Thus impaired signaling via FGFR1c in the adult ß-cell leads to both aberrant glucose sensing and impaired insulin processing that together ultimately progress to diabetes development.

Example 7. Demonstration that *Ipf1/Pdx1* is required for the expression of FGFR1 and FGFR2. The disorganisation of islets and the down-regulation of Glut2 observed in the *Ipf1/dnFGFR1c* mice resembles the islet phenotype associated with a ß-cell specific activation of the transcription factor *Ipf1/Pdx1* demonstrated in the *RIP1$^{\Delta}$/Ipf1* mice previously established in our laboratory (Ahlgren et al., 1998). These mice develop diabetes at 10-15 weeks of age due to a Cre-mediated excision exon 2 of the *Ipf1/Pdx1* gene. Analysis of pancreas derived from *RIP1$^{\Delta}$/Ipf1* mice showed that the expression of FGFR1 and FGFR2 was down-regulated in the ß-cells (Figure 5 and data not shown). Some residual expression could still be observed which probably reflects a residual Ipf1 expression in some of the ß-cells, since these mice develop diabetes at a stage when approximately 20% of the ß-cells still express Ipf1 (Ahlgren et al., 1998). To investigate whether the decrease in FGFR1c expression in the *RIP1$^{\Delta}$/Ipf1* mice might lead to a perturbed insulin processing we performed an analysis of the expression of the prohormone convertases in the *RIP1$^{\Delta}$/Ipf1* mice. PC1/3 was severely down-regulated in these mice as well (Figure 5). The *RIP1$^{\Delta}$/Ipf1* mice also displayed an increased proinsulin content in their ß-cells coupled with a decreased levels of C-peptide 1 and 2 (Figure 5, and data not shown).

In combination these results provide evidence that Ipf1 is required for the expression of FGFR1 in the adult ß-cells,

thereby ensuring a high level of Glut2 and PC1/3 expression. Thus, both perturbed Ipf1 expression and impaired signalling via FGFR1c leads to diabetes due to impaired glucose sensing and insulin processing in the adult ß-cells with diabetes manifestation as a consequence. These results suggest that signalling via FGFR1c is critical for the maintenance of a mature, functional ß-cell phenotype, and that *Ipf1/Pdx1* by virtue of its key role in controlling, directly or indirectly, many aspects of the ß-cell glucose homeostasis machinery is pivotal for the ß-cell's capacity to preserve normoglycemia.

Example 8. Analysis of pancreas from diseased type 2 diabetics and control individuals. Double immunohistochemical analyses of pancreas derived form non-diabetic humans show that both FGFR1 and FGFR2 are selectively expressed also in human insulin-producing β-cells and not glucagon producing α-cells (Figure 6a and 6b). Similar to mice with diabetes due to impaired FGFR1-signalling [5,6], type 2 diabetics display disorganized islets with glucagon-producing α-cells mixed with the insulin-producing β-cells (Figure 6d), whereas non-diabetics have normal islets with glucagon-producing cells surrounding the core of insulin-producing β-cells (Figure 6c).

Moreover, the expressions of both FGFR1 and FGFR2 are drastically reduced in the insulin-producing β-cells of type 2 diabetics as compared to that of control individuals (Figure 7). Consequently, as has been shown for mice with impaired FGFR1 signalling, type 2 diabetics show reduced expression of PC1/3 in their β-cells (Figure 8b, 8e) whereas the expression of PC2 appear less affected (Figure 8a, 8d). The reduction of PC1/3 expression is paralleled by an

increase in β-cell proinsulin content in the Type 2 diabetics as compared to the control individuals (Figure 8c, 8f).

These experiments demonstrate that β-cells of type 2 diabetic patients have disorganized islets, reduced expression of FGFR1, FGFR2 and PC1/3 as well as an increased proinsulin content in their β-cells. These findings when combined provide evidence that, similar to mice with diabetes due to an impaired FGFR1-signalling in adult β-cells, attenuation of FGFR1-signalling pathway in human β-cells is coupled to diabetes. We suggest that FGF-signalling in the adult pancreas ensures a functional β-cell identity and glucose homeostasis. Thus an impaired expression, or activity, of components within the FGF-signalling pathway is coupled to diabetes in both mice and humans. In both mice and humans impaired FGF-expression and signalling is coupled to a decrease in PC1/3 expression. The decrease in PC1/3 in turn leads to a perturbed processing of insulin with elevated pro insulin levels as a result. Consequently the FGF-signalling pathway, i.e. including components upstream and downstream of the FGFR-ligand interaction, is a suitable target for the development of new therapies to cure diabetes.

We have also recent data from analyses of expression of Id-proteins [Norton, 2000] that Id2 and Id3 are targets downstream of FGFR1-signalling. As is shown in Figure 9, both Id2 and Id3 are normally expressed in pancreatic endocrine cells (Figure 9a, 9d). However, in mice with diabetes due to attenuation of FGF-signalling in β-cells, i.e. the FRID1 and *RIP1/Ipf1*$^{\Delta}$ mice, the expression of both Id2 (Figure 9b, 9c) and Id3 (Figure 9e, 9f) are severely down-regulated. These results provide evidence that Id2 and Id3 expression in β-cells are dependent of FGF-signalling and hence represent

downstream components of the FGF-signalling pathway in adult β-cells. Id2 and Id3 thus represent candidate targets for the development of new therapies to cure diabetes.

FIGURE LEGENDS

Fig. 1: **(A-F)** Analysis of FGFR1 expression in adult pancreas showing that FGFR1-expression **(B,D)** coincides with insulin **(C)** but not glucagon **(A)** expression. **(E,F)** In *Ipf1/dnFGFR1c* transgenic mice the dnFGFR1c protein **(F)** is highly expressed in $IPF1^+$ cells **(G)** as detected by antibodies against the Rat IgG Fc region that is coupled to the dnFGFR1c domain.

Fig. 2: **(A)** Analysis of number Ins^+-cells over number of $Isl1^+$-cells showing a 30% decrease in the number of Ins^+-cells in *Ipf1/dnFGFR1c* (TG) mice as compared to wild-type (wt) mice. Data from at least 4 independent mice, n = 7435 cells. **(B)** Measurement of total pancreatic insulin content (µg insulin/mg pancreas protein) from non-fasted animals (n = 6 wt and n = 8 TG) show that the 30% decrease in number of Ins^+-cells **(A)** is accompanied by a 28% decrease in total pancreatic insulin content in the *Ipf1/dnFGFR1c* mice as compared with their age-matched wild type littermates. **(C)** The 30% decrease in number of insulin cells results in a relative 20% increase in number of Glu^+-cells in *Ipf1/dnFGFR1c* mice. Data from at least 4 independent mice, n = 7525 cells.

Fig. 3: **(A-D)** Confocal microscopy analyses of insulin **(C,D)** and glucagon **(A,B)** expression in wild-type **(A,C)** and *Ipf1/dnFGFR1c* transgenic **(B,D)** mice show that the islet organisation in the transgenic mice is abnormal but that there is no co-expression between insulin and glucagon within the islet cells. **(E-H)** Expression of glucose sensing and insulin processing enzymes is impaired in *Ipf1/dnFGFR1c* mice.

Images show that Glut2-expression **(E,F)** in adult β-cells is lost as a result of *Ipf1/dnFGFR1c* expression and that only a very low level of PC3 expression **(G,H)** remains in *Ipf1/dnFGFR1c* mice, and preferentially in α-cells.

Fig. 4: Analyses of insulin variants in pancreas from wild-type **(A, C, E, G)** and *Ipf1/dnFGFR1c* **(B, D, F, H)** mice using C-peptide 1 **(A and B)**, C-peptide 2 **(C and D)**, insulin **(E and F)** and proinsulin (**G** and **H**) anti-sera. **(A, C, E, G)** In wild-type pancreas insulin is present predominantly in its fully processed form **(A, C, E)** with virtually no detectable unprocessed pro-insulin **(G)**. Note that the fluorescence in **C** represents background, non-β-cell reactivity due to the use of mouse monoclonal anti-sera. **(B, D, F, H)** *Ipf1/dnFGFR1c* mice display a perturbed proinsulin processing resulting in reduction of fully processed insulin **(B, D, F)** while a substantial, readily detectable fraction remains in the form of proinsulin **(H)**.

Fig. 5: **(A-F)** Loss of IPF1/PDX1 activity in β-cells (Ahlgren et al. 1998) results in a drastically reduced FGFR1 **(A,B)** and PC3 **(C,D)** expression. This loss of PC3 expression results in perturbed proinsulin processing, with a reduction in detectable levels of fully processed insulin (not shown) accompanied by increased levels of detectable proinsulin **(E,F)** within the β-cells of the *Rip1/Ipf1*$^{\Delta}$ mice **(B,D,F)** as compared to wild-type littermates **(A,C,E)**.

Fig. 6: Expression of FGFR1 and FGFR2 in adult human islets. Confocal microscopy analyses of FGF receptor and glucagon expression in adult human pancreas showing that the receptors FGFR1 (**a**) and FGFR2 (**b**) are expressed in □-cells and not in β-cells. The islets of human type 2 diabetic patients (n=3) (**d**) are disorganized with glucagon producing cells found scattered throughout the islets, as compared with non-diabetic pancreatic tissue where the glucagon cells are found at the periphery of the islet (**c**).

Fig. 7: Impaired expression of FGFR1 and FGFR2 in human type 2 diabetic patients. Immunohistochemical analyses demonstrate that both FGFR1 (**a** and **c**) and FGFR2 (**b** and **d**) are expressed at clearly reduced levels in β-cells of patients (n=3) suffering from type 2 diabetes. Non-diabetic pancreatic tissue (**a** and **b**), diabetic pancreatic tissue (**c** and **d**).

Fig. 8: Impaired expression of PC1/3 in human type 2 diabetic patients. Immunohistochemical analyses show that PC2 (**a** and **d**) expression is unaffected in human type 2 diabetic patients (n=3), whereas expression of PC1/3 (**b** and **e**) is drastically reduced. The down-regulation of PC1/3 expression leads to functional impairment of insulin processing as revealed by the increase in intracellular proinsulin content (**c** and **f**). Non-diabetic pancreatic tissue (**a-c**), diabetic pancreatic tissue (**d-f**).

Fig. 9: Id2 and Id3 are downstream of FGFR1c-signalling. Id2 and Id3 down-regulated in β-cells of overt diabetic FRID1 and *RIP1/Ipf1*$^{\Delta}$ mice. Both Id2 and Id3 are normally expressed in mouse adult islets cells (**a,d**). The expression of Id2 (**a-c**) is greatly reduced in β-cells of diabetic FRID1 (**b,e**) and *RIP1/Ipf1*$^{\Delta}$ (**e,f**) mice as compared to that of wild type littermates and the expression of Id3 (**d-f**) virtually absent **(e,f)** in these diabetic mouse models. The remaining Id2 and Id3 expression still observed in both the FRID1 and *RIP1/Ipf1*$^{\Delta}$ islets represent expression in scattered glucagon cells.

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C L A I M S

1. A transgenic diabetes type II model laboratory animal comprising ß-cells expressing a dominant negative form (dnFGFR1c) of FGFR1c.

2. The transgenic animal of claim 1 or 2, wherein the animal is a mouse.

3. Use of the *Ipf1/Pdx1* promoter for controlling expression of FGFR1c.

4. ß-Cells in which the expression of PC1/3 is down-regulated or absent.

5. ß-Cells competent to express a dominant negative form (dnFGFR1c) of FGFR1c.

6. The ß-cells of claim 4 or 5 comprised by an adult pancreas.

7. Mature ß-cells incompetent to express Glut2.

8. The ß-cells of claim 7 comprised by an adult pancreas.

9. Mature ß-cells in which the processing of proinsulin to insulin is substantially impaired.

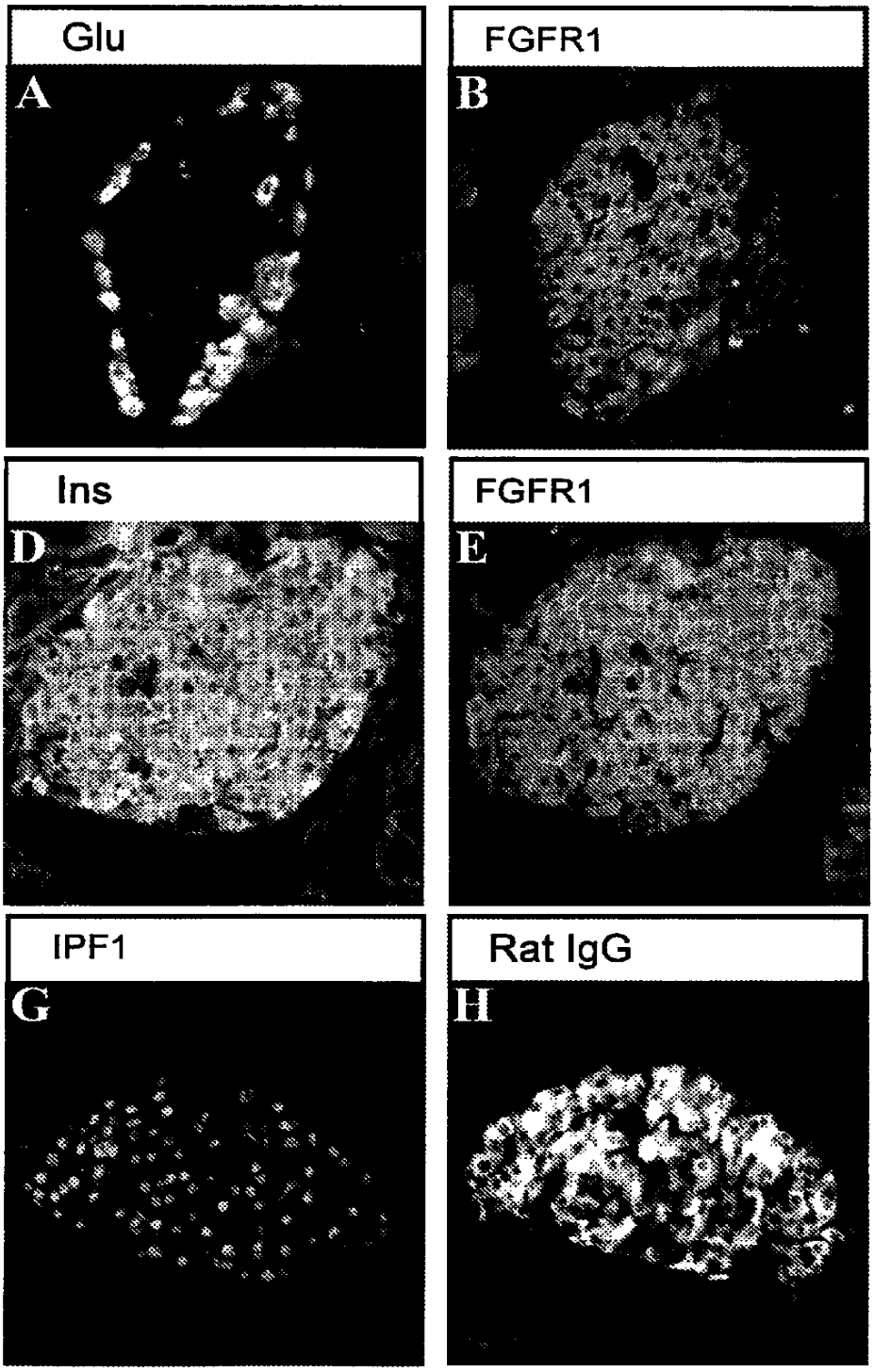
10. The ß-cells of claim 9, wherein levels of proinsulin convertase are substantially reduced in comparison with such levels in non-transgenic mice.

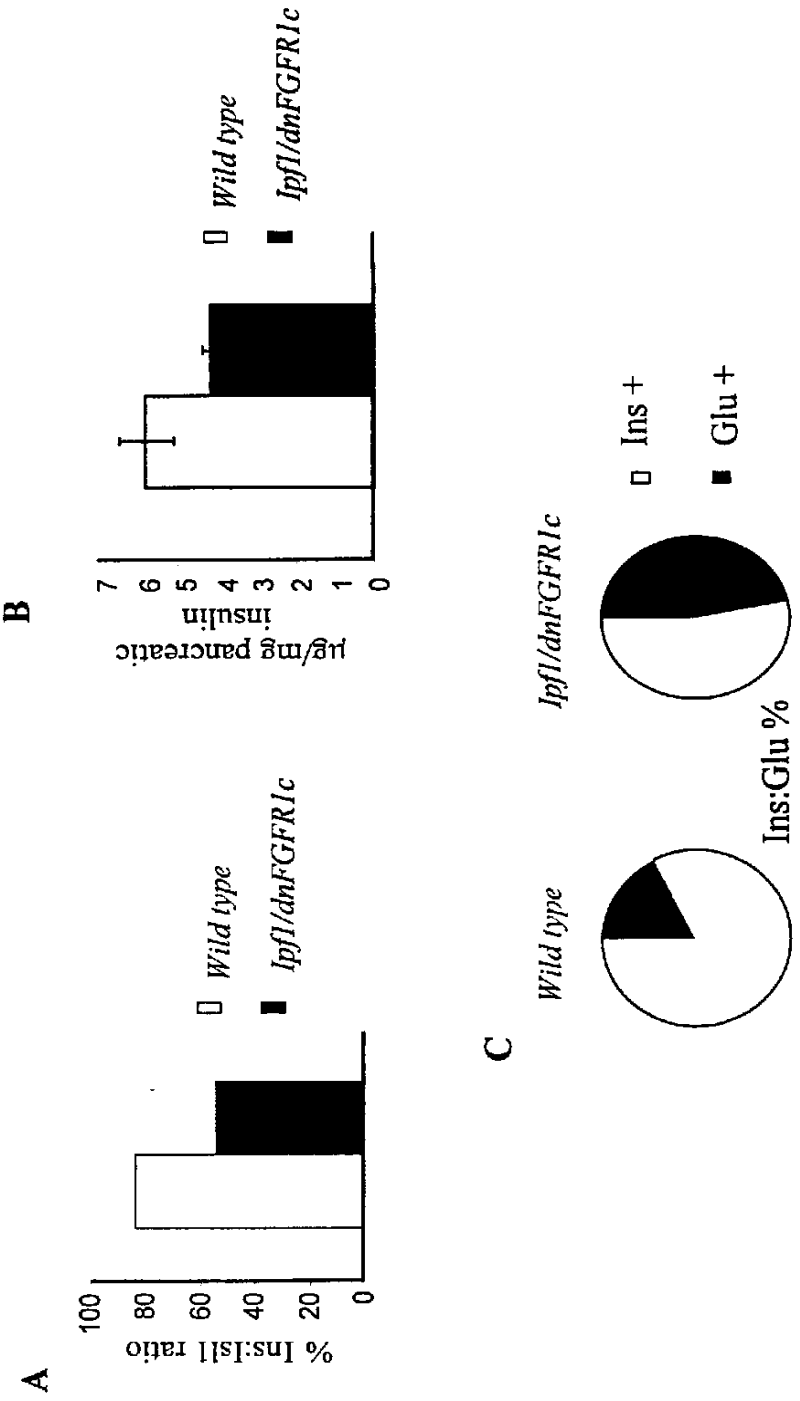
11. The ß-cells of claim 9 comprised by an adult pancreas.

12. A method of preventing or treating type II diabetes in a person by administering to said person a pharmacologically effective amount of an agent that activates the FGF signalling pathway in pancreatic ß-cells promoting the formation of proinsulin convertase and Glut2.

13. A method of preventing or treating type II diabetes in a person by administering to said person a DNA fragment that allows expression of Ipf1 in pancreatic ß-cells which promotes the formation of FGFR 1, proinsulin convertase and Glut2.

Fig. 1




A
% Ins:Isl ratio
100
80
60
40
20
0
Wild type
Ipf1/dnFGFR1c
B
μg/mg pancreatic insulin
7
6
5
4
3
2
1
0
Wild type
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C
Wild type
Ipf1/dnFGFR1c
Ins +
Glu +
Ins:Glu %

Fig. 2

Fig. 3

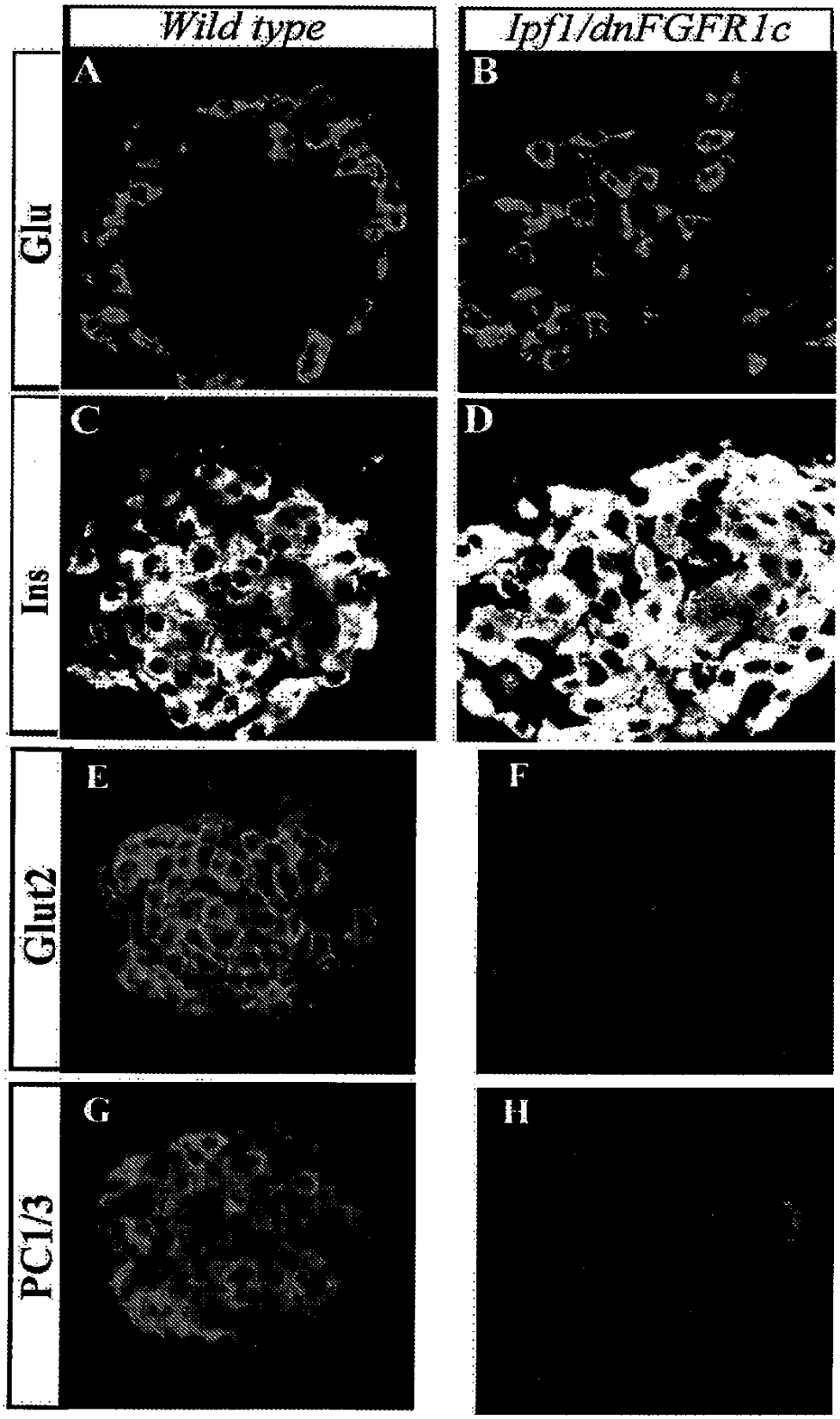


Fig. 4

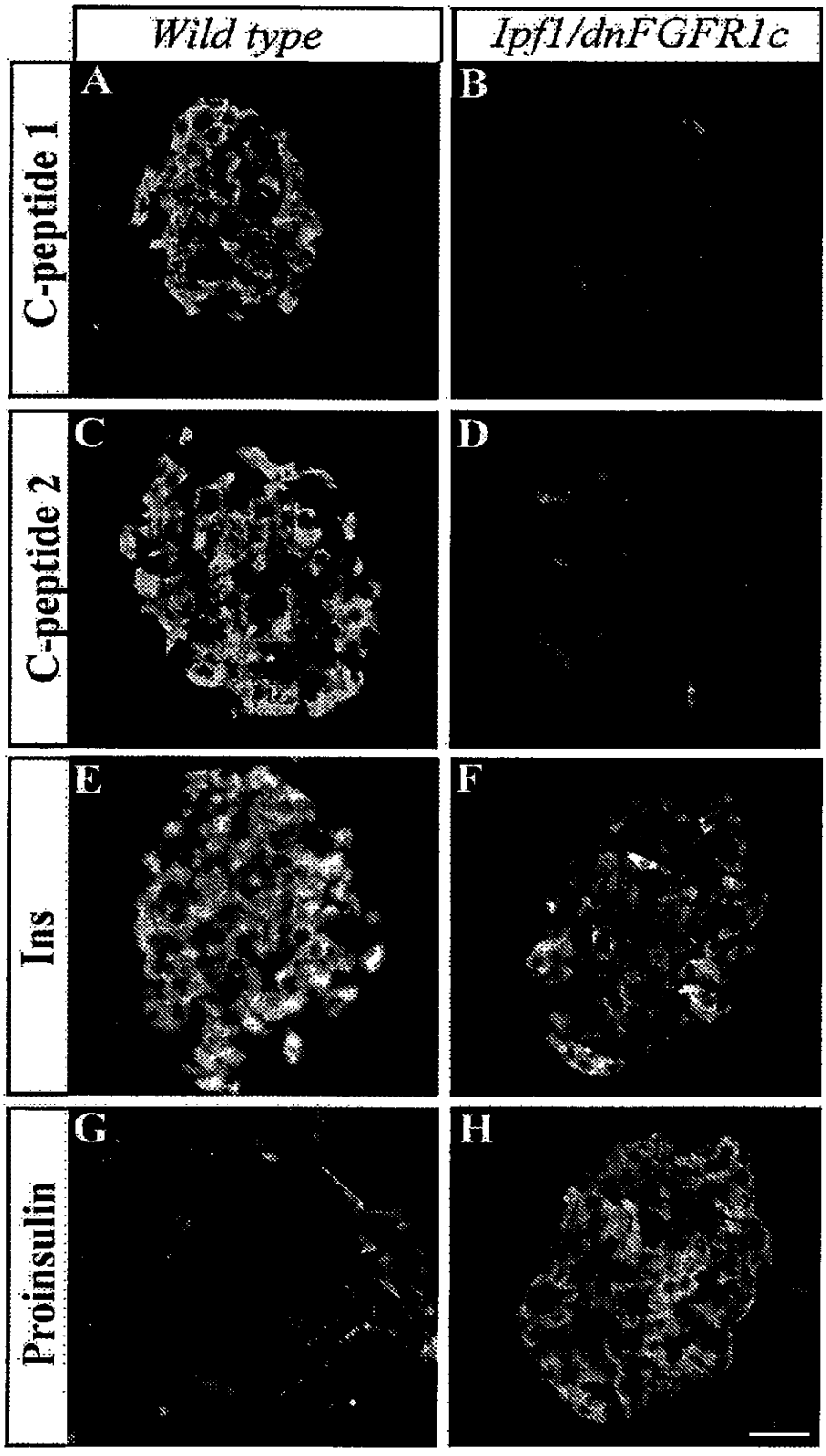

Wild type
Ipf1/dnFGFR1c
C-peptide 1
C-peptide 2
Ins
Proinsulin
A
B
C
D
E
F
G
H

Fig. 5

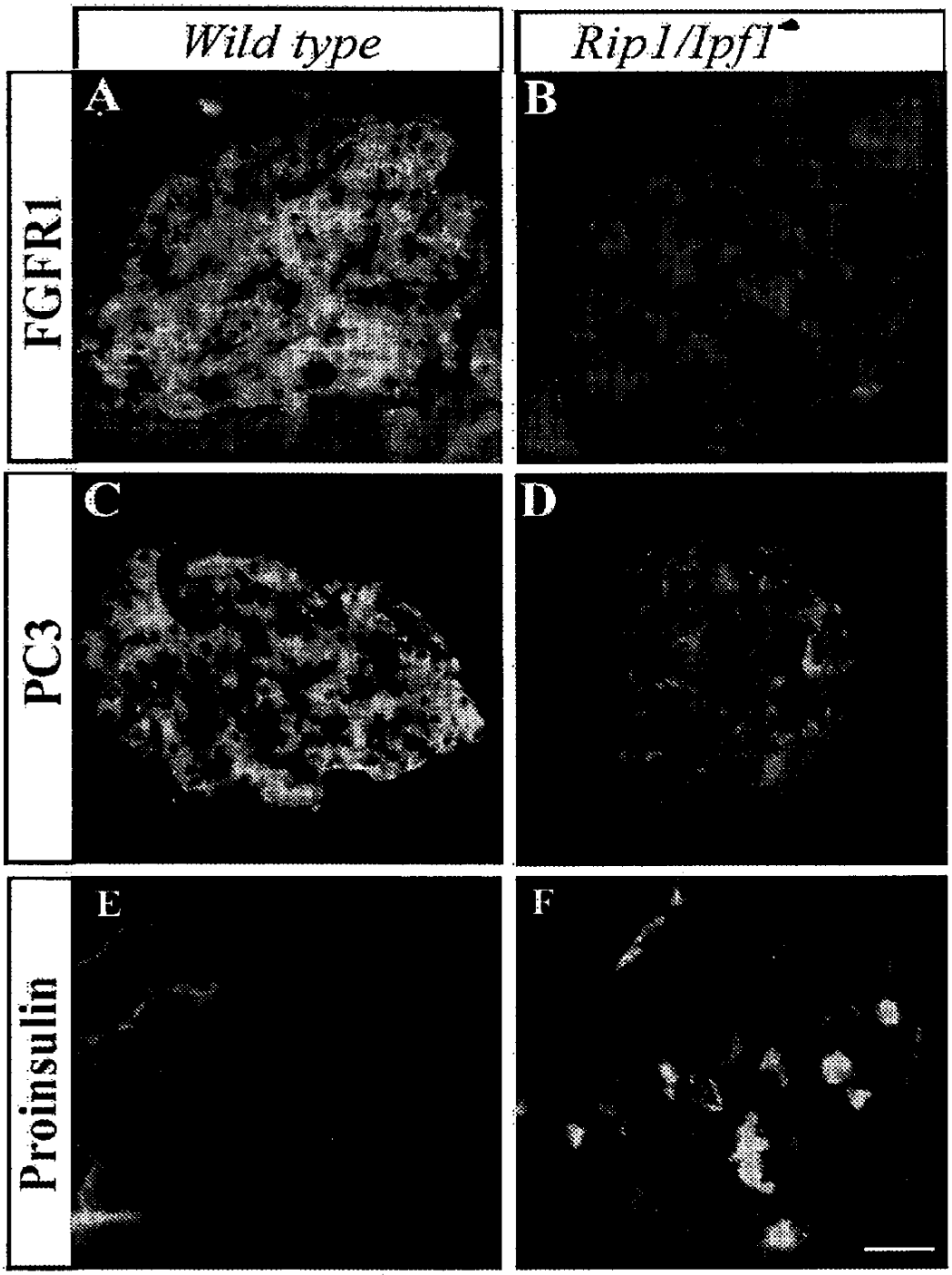
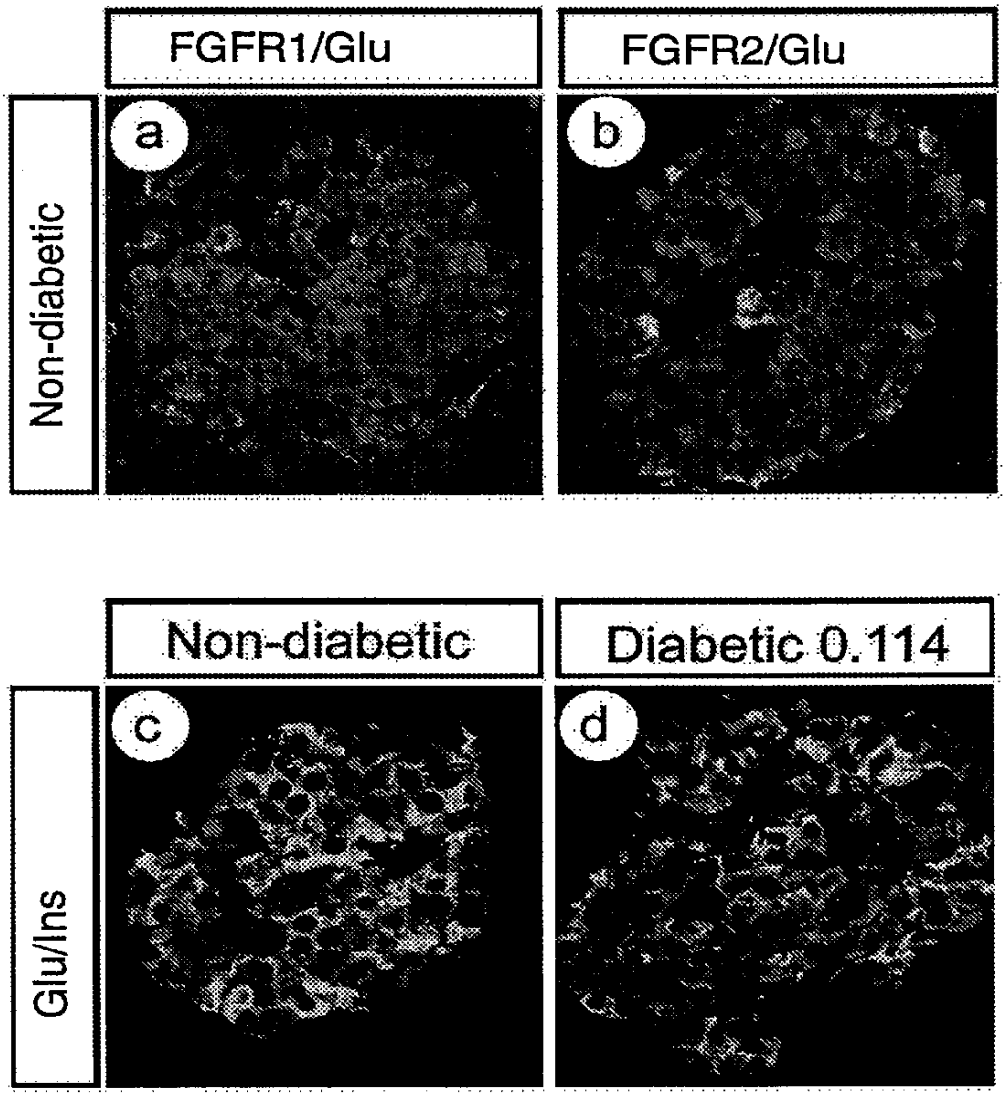


Fig. 6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FGFR2/Glu
Non-diabetic
a
b
Non-diabetic
Diabetic 0.114
Glu/Ins
c
d

Fig. 7

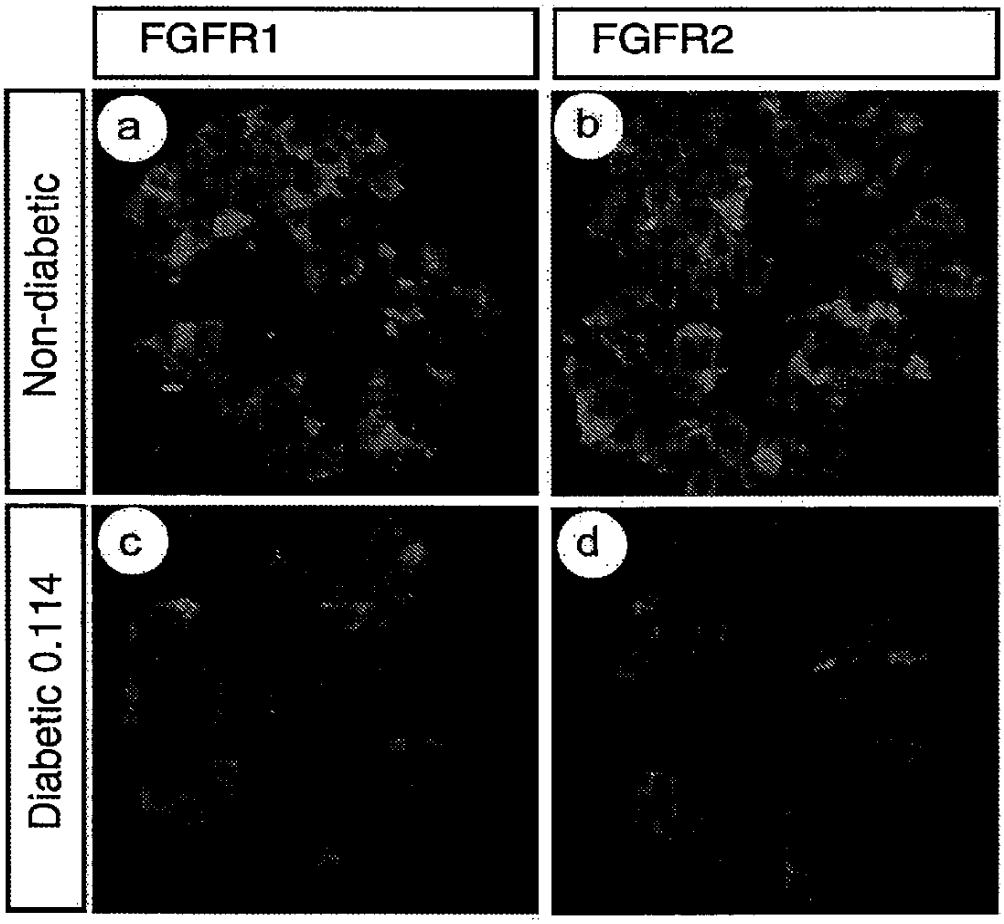


Fig. 8

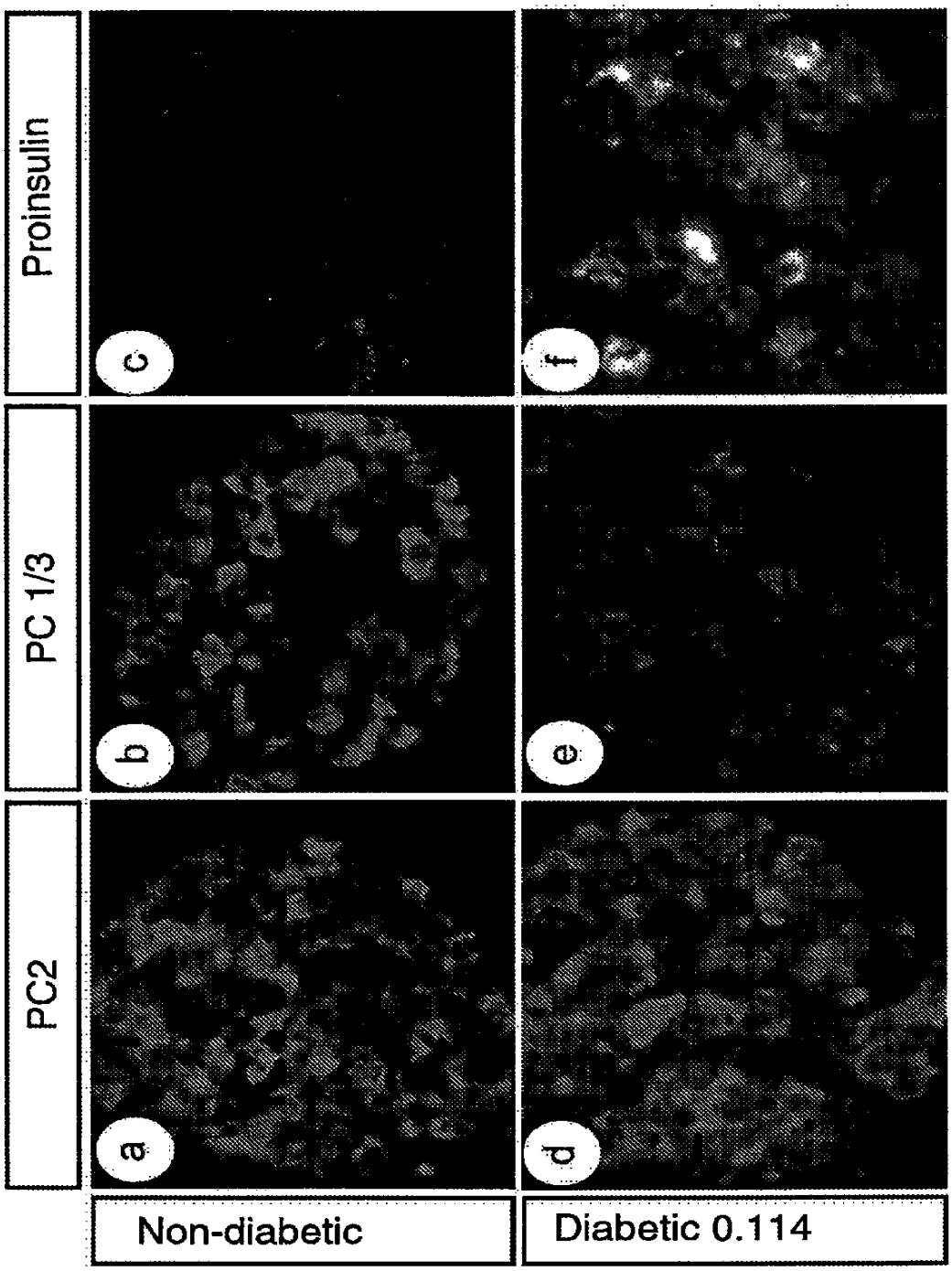
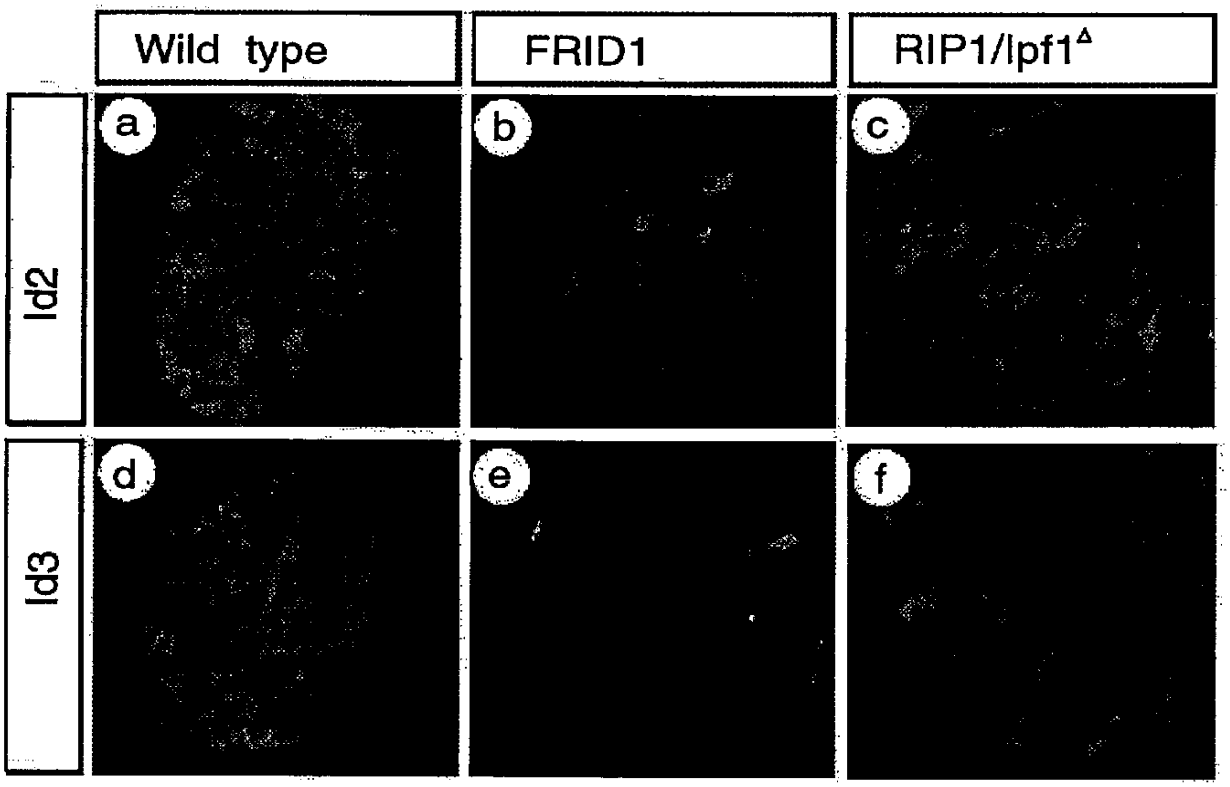


Fig. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/00783

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A01K 67/027, C12N 15/63, C12N 5/10
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A01K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	The Journal of Histochemistry & Cytochemistry, Volume 45, No 7, 1997, Sian E. Hughes, "Differential Expression of the Fibroblast Growth Factor Receptor (FGFR) Multigene Family in Normal Human Adult Tissues" page 1005 - page 1019	5-6
A	--	1-3
X	The Journal of Clinical Investigation, Volume 104, No 2, 1999, Katleen Hostens et al, "Exposure of human islets to cytokines can result in disproportionately elevated proinsulin release" page 67 - page 72	4,9-11
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 2001	29 -08- 2001
Name and mailing address of the ISA Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/EÖ Telephone No. +46 8 782 25 00

Form PCT ISA 210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 01/00783

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	The EMBO Journal, Volume 17, No 6, 1998, Giulia Celli et al, "Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning" page 1642 - page 1655	5-6
A	--	1-3
X	Genes & Development, Volume 12, 1998, Ulf Ahlgren et al, "Beta-Cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the Beta-cell phenotype and maturity onset diabetes" page 1763 - page 1768	7-8
P,X	-- Nature, Volume 408, December 2000, Alan W. Hart et al, "Attenuation of FGF signalling in mouse Beta-Cells leads to diabetes" page 864 - page 868	1-11
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE01/00783

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE01/00783

According to Article 34 (3) (a-c) and Rule 13.2, an international application shall relate to one invention only or to a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over the prior art.

The application is considered to contain the following independent inventions:

Invention 1, claims 1-3 and 5-6: A transgenic animal and a ß-cell that encode a dominant negative form of FGFR1c, and the use of a vector containing a Ipf1/Pdx1 promoter expressing FGFR1c.
Invention 2, claims 4 and 9-11: ß-cells where convertion of proinsulin to insulin is decreased, by for example down regulation of PC1/3.
Invention 3, claims 7-8: ß-cells that do not express Glut2.
Invention 4, claims 12.
Invention 5, claims 13.